

The University of Chicago

**FACTORS AFFECTING REGENERATION
OF THE HORSERADISH ROOT**

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
1939

By
ROBERT C. LINDNER

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ROBERT C. LINDNER

(WITH TWELVE FIGURES)

Introduction

The term "regeneration" is ordinarily used to indicate the formation anew of a part of an organism that has been lost. In this paper the term is applied to the initiation of buds and roots from isolated pieces of the horseradish root. The growth of any plant axis, whether it be root or stem, may be separated into at least two phases: (1) The actual initiation of a growing point from a mass of meristematic tissue, and (2) the maintenance and subsequent development of this growing point. In the work reported here, no attempt was made to analyze the factors involved in the subsequent development of buds and roots after they were initiated. Reviews of the older literature on regeneration may be found elsewhere (9, 12, 15, 16).

The horseradish, *Cochlearia armoracia*, is a convenient plant for a study of this type, because small isolated pieces of the root regenerate both buds and roots quite readily when placed in the proper environment. Furthermore, these pieces are fairly resistant to invasion by microorganisms. Previous work (13) has shown that normally the initiation of buds and roots is confined to those regions of the root where a lateral root has been broken off. Both buds and roots apparently arise from identically the same region. It has also been shown that the application of relatively high concentrations of indoleacetic and naphthyleneacetic acids inhibits the development of buds and stimulates the development of roots. The purpose of this paper is to elucidate some of the factors involved in the initiation of buds and roots, with particular emphasis on bud initiation.

Methods

Horseradish roots were obtained from a market in Chicago and stored at 2°-4° C. until used. If the humidity of the storage room is kept high

¹ Contributions from the Hull Botanical Laboratory no. 513.

enough to prevent excessive desiccation, the roots may be stored for months at this temperature without apparent detrimental results. Roots of approximately the same diameter (20–28 mm.) were used to insure more uniform test material. When a root was prepared for use, the upper and lower portions were discarded, leaving the central portion for experimental purposes (fig. 1). Adhering dirt was removed by scrubbing with a stiff-bristled brush



FIG. 1. Stages in preparation of horseradish root: (1) dormant root; (2) after trimming and washing; (3) after removal of test pieces. The ends of the root traces are visible as small elevations on the surface of the root.

while washing thoroughly with water. Care was taken to prevent injury to the root during this process. With a short cork-borer, a small piece of root was removed containing a root trace and its surrounding tissue. The piece was removed from the cork-borer by pressing on the end opposite the periderm with a glass rod (fig. 2A). A short cork-borer is advantageous in this procedure; the one used in most of the work was 6 mm. in diameter and 30

mm. long. After removal from the cork-borer, a test piece of the desired length (usually either 1 mm. or 3 mm.) was cut with a sharp razor, and the remaining tissue discarded. Ten such test pieces were placed with cut ends down on clean moist sand in a Petri dish (fig. 2B) and kept in the dark at

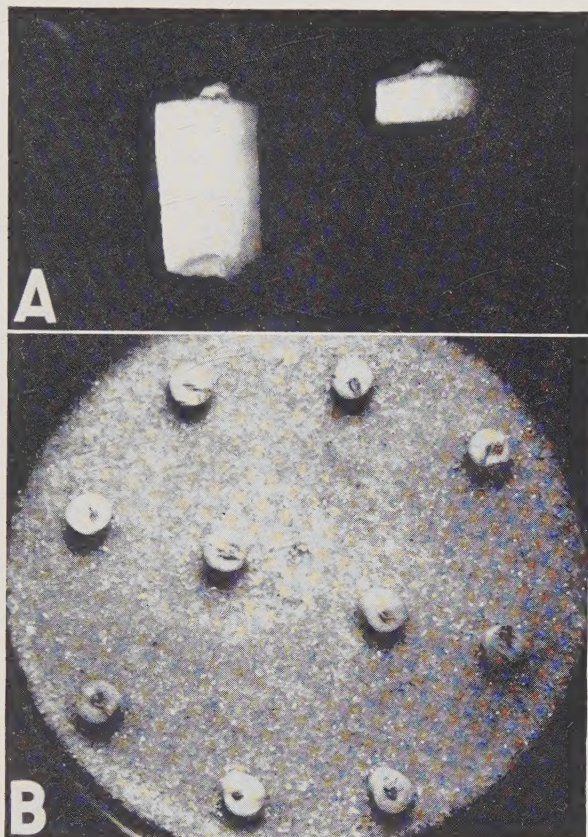


FIG. 2. Preparation of test pieces. A: piece on left as removed from cork-borer; piece on right trimmed to 3 mm, test piece ($\times 2$). B: test pieces on moist sand in Petri dish. The ends of the root traces are visible as small elevations on the surface of the pieces (natural size).

25° to 26° C. in an electric oven. The sand used in this work was washed successively with concentrated hydrochloric acid, tap water, and distilled water; it was then dried before use. About 23 ml. of dry sand were added to each Petri dish—an amount just sufficient to take up by capillarity 10 ml. of distilled water (or the solution to be tested). Under these conditions buds and roots first appear in about six days, and by nine days the maximum

number is formed. Observations were usually made after ten days with the aid of a dissecting microscope (fig. 3).

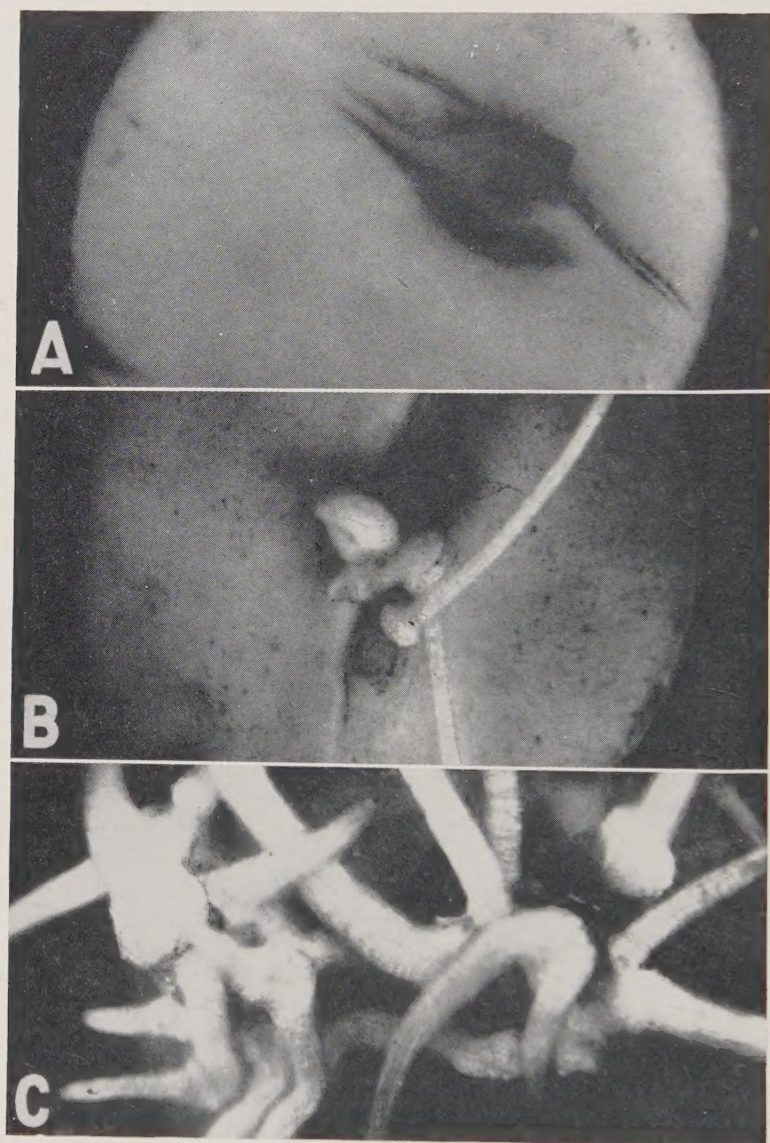


FIG. 3. Regeneration of test pieces. A: test piece at time of preparation. B: normal regeneration from test piece 3 mm. in length showing 2 roots and 3 buds. C: test piece treated with a 1 per cent. lanolin mixture of naphthylacetic acid showing formation of numerous roots ($\times 15$).

Since a bud or root is discernible at the surface of the test piece as soon as it becomes organized, the number of buds and roots formed may be used as a quantitative measure of regeneration. The number produced per ten test pieces was chosen as a standard measure, each Petri dish thus constituting a sample. Five replications or more were used, giving a population of at least fifty test pieces for any single determination. No attention was given to the relative size or vigor of an individual bud or root since only the factors involved in their initiation were being considered. The results reported in this paper were obtained from over 15,000 test pieces.

A major criticism of the method used was the lack of control of micro-organisms. Most of the test pieces were able to resist invasion, however, if they were not damaged severely by the experimental treatment. Since the irregular surface of the piece made sterilization almost impossible, it was thought better to use a larger number of test pieces rather than introduce other variables in trying to obtain sterile cultures. Another problem was the introduction of chemical impurities in the sand which might affect the growth responses of the pieces. Such possible effects were minimized by thoroughly washing the sand with acid and water before use. No satisfactory substitute for sand was found that afforded adequate aeration and a good water supply. An additional problem was the past history of the material tested. If one were to grow the horseradishes under controlled conditions, take special precautions to see that they receive no injuries, and then rigorously select the roots, more uniform material would be obtained. Such a procedure is not feasible, and thus the best alternative appears to be the selection of roots of about the same size and the use of only those root traces that appear to be comparable. At any rate, it is not the actual number of buds and roots produced in any one test that is significant; it is the general trend of an experiment which results from a large total number of tests.

TABLE I

RELATION OF AREA OF TEST PIECE TO AMOUNT OF REGENERATION

DIAMETER	AVERAGE NUMBER OF ROOTS PER TEN PIECES	AVERAGE NUMBER OF BUDS PER TEN PIECES
<i>mm.</i>		
3	16 ± 2*	33 ± 2*
6	19 2	32 2
9	17 2	35 2
12	39 3	70 4
15	37 3	67 4

* Standard deviation expressed to nearest whole number.

$$\sigma = \sqrt{\frac{\sum d^2}{n-1}}$$

Experimentation

As a preliminary experiment, it was essential to determine the effect of the diameter of the test piece on initiation of buds and roots. Pieces 3 mm. long, varying in diameter from 3 mm. to 16 mm., were tested in the dark at 25° C. Owing to the distribution of the root traces in the root, only one root trace was included per test piece in pieces up to 9 mm. in diameter; above 9 mm. two root traces had to be included. The results, shown in table I, tend to indicate that only the root traces are important in bud and root initiation. For convenience in handling, the 6-mm. piece was chosen as the standard piece.

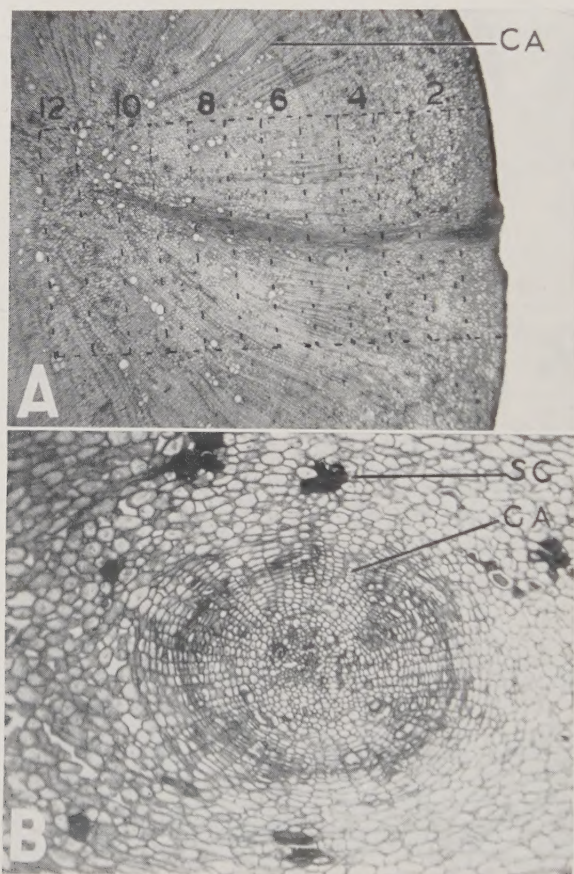


FIG. 4. Tissues associated with test piece. A: cross section of root 10 mm. in diameter showing longitudinal section of root trace. Diagram of test pieces imposed and drawn to scale as if the root were 24 mm. in diameter; ca, cambium ($\times 15$). B: tangential section through phloem portion of root showing cross section of root trace; ca, cambium; sc, stone cell ($\times 150$).

It was also necessary to know the relation of length of test piece to amount of regeneration. Pieces 6 mm. in diameter, but of lengths varying from 1 to 12 mm., were tested in the dark at 25° C. Figure 4 shows the tissues involved in these tests and the results are shown in figure 5. Al-

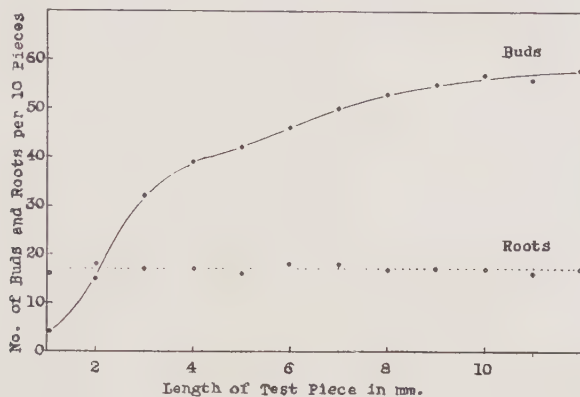


FIG. 5. Relation of length of test piece to amount of regeneration.

though the inner tissues apparently have no effect on the number of roots initiated, they do have a marked effect on the number of buds initiated.

Further evidence concerning the importance of the root trace for bud initiation was obtained by the following experiment. Pieces 6 mm. in diameter and 8 mm. long were used, and the root trace was severed by cutting a deep notch 1 mm. from the periderm. Others were ringed in the same place in order to sever nearly all tissue except the root trace. In all cases where the root trace was cut, the amount of regeneration corresponded to that of a piece 1 mm. long. On the other hand, when a root trace was left intact, the amount of regeneration corresponded to that of a piece 8 mm. long. In another experiment, pieces of various sizes were taken from parts of the root in which there were no root traces and tested in the dark at 25° C. In no case was there initiation of either roots or buds.

The question arose as to whether an inner portion of the test piece would initiate buds and roots if it were separated from the outer portion containing the periderm. Standard pieces 6 mm. in diameter and 8 mm. long were used; the outer 1 mm. consisting of periderm and outer phloem was discarded. In some cases the tissue produced a root or bud, more frequently a bud. If the outer 2-mm. piece was discarded, only buds were initiated, and these very rarely. These results seem to indicate that there is some factor, or factors, for both root and bud initiation in the outer 1 or 2 mm. of tissue.

It appears, therefore, that root and bud initiation are confined to the root trace. Only the outer tissues seem to be necessary for root initiation; for

maximum bud initiation, however, both inner and outer tissues seem to be necessary. Hereafter, for the sake of simplicity, the influence of the inner tissues in the stimulation of bud initiation will be referred to as the "bud factor." Furthermore, the term "factor" will be used in a broad sense to include any agent (physical, chemical, or otherwise) that produces an effect, and will not necessarily imply that a chemical entity is involved.

After the "bud factor" was shown to be confined to the root trace, it became desirable to know something about the rate of movement. Standard pieces 6 mm. long were divided into ten different lots. From one lot each day, the outer 1 mm. of tissue was removed from the pieces, and the inner 5 mm. were discarded. Observations were made on the fifteenth day. The results shown in figure 6 indicate that there is very little movement of the

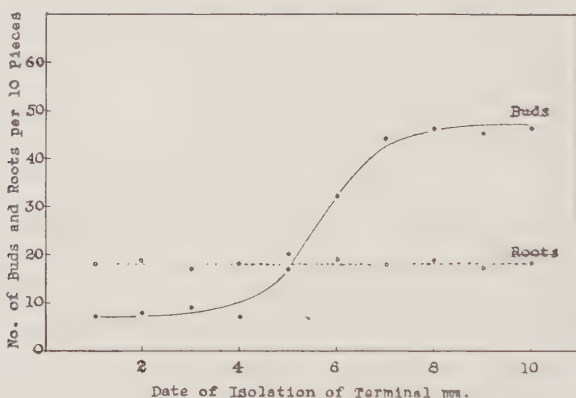


FIG. 6. Relation of time of isolation of 1 mm. test piece to amount of regeneration. The abscissa indicates time of isolation in days after beginning of experiment.

"bud factor" during the first four days of the experiment. Between the fifth and seventh days, however, there is a rather sudden movement. There are several ways of explaining these results. We may assume the "bud factor" to be present in the intact root, but we must then conclude that it does not move toward the periderm until at least four days after isolation of the test piece. On the other hand, the "bud factor" may not be present in the intact root and may be formed only about four days after isolation of the test piece. Another possibility is that the inner tissues merely act as a reservoir for the accumulation of bud inhibitors that are present in the outer tissues. These inhibitors may not move inward until after four days.

Since the "bud factor" has been shown to be transported along the root trace, some information regarding the diffusibility of this stimulus was sought. Standard test pieces 8 mm. long were used and the outer 1 mm. was removed with a clean, sharp razor. In some cases the two resulting pieces were immediately pressed tightly together, while in others a layer of

gelatin, agar, lanolin, or lens paper was placed between them. In every case the 1-mm. piece acted like an isolated 1-mm. piece. Apparently there was no diffusion of the "bud factor" across the break in continuity. These experiments were duplicated by attaching to the 1-mm. test piece a piece of inner tissue 7 mm. in length which had been killed with ether vapor (ether removed by aeration for six hours before pieces were used). In every case the 1-mm. test piece was killed, probably by the mustard oil or by other decomposition products of the dead tissue. There remained the possibility that if the "bud factor" were not present when the test piece was first isolated, it might not form unless it was under the influence of the developing primordia in the 1-mm. test piece. To test this possibility, the above experiments were duplicated, using test pieces that were severed 1, 2, 3, and 4 days after isolation. The same results were obtained, however, and in no case was there any evidence of bud stimulation. Thus one may conclude that the "bud factor" requires intact cells for its transfer.

Previous work has shown that when the horseradish root is cut into transverse segments about 4 cm. long, the development of buds and roots occurs in a polar manner; *i.e.*, buds develop at the morphological upper ends of the segments and roots at the morphological lower ends, regardless of the orientation of the segments during the period of regeneration (13). These observations were limited to gross development of both buds and roots and were not concerned with the actual initiation of growing points. Since there is this polarity in development, there might also be a polarity in the distribution of factors responsible for initiation. To test this possibility, transverse segments of the root, about 6 cm. long, were placed in a moist chamber on moist sand and maintained in the dark at 25° C. For nine successive days, standard test pieces, 6 mm. in diameter and 3 mm. in length, were removed from the tops and bottoms of these segments. Observations were made on the twelfth day. The results are shown in figure 7. The intact root appar-

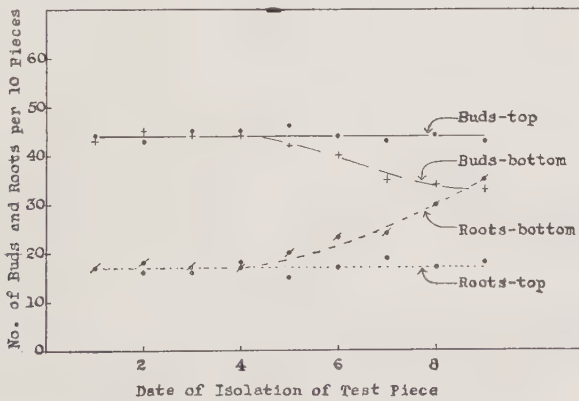


FIG. 7. Relation of polarity to initiation of buds and roots. The abscissa indicates time of isolation in days after beginning of experiment.

ently possesses no polarity in the distribution of the factors for bud and root initiation. The gradual increase in the number of roots and decrease in the number of buds, from the test pieces removed from the lower portions of the root segments, could be interpreted on the basis of the gradual accumulation in this region of some factor that stimulated root initiation and inhibited bud initiation. This factor might consist of the native auxins.

A partial analysis of the internal environment affecting bud and root initiation has shown that the inner tissues of the root trace stimulate bud initiation. This "bud factor" is transported along the root trace, is not diffusible, and is not polarly distributed. There are indications that it may be inactivated by one of the factors controlling root initiation.

Some of the relationships of the external environment to regeneration were next investigated. In order to determine the effects of variations in temperature, standard test pieces 6 mm. in diameter and 3 mm. in length were kept at various temperatures in the dark. Observations were made at the end of ten days. The results are shown in figure 8. The optimum

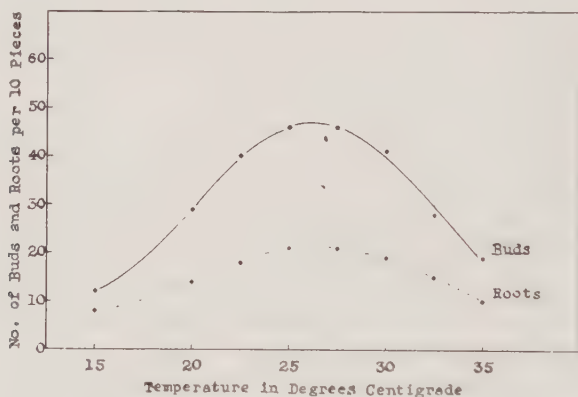


FIG. 8. Effect of temperature on bud and root initiation.

temperature for both root and bud initiation is about 26° C. Between 15° C. and 25° C. the Q_{10} for bud initiation is 2.8, while that for root initiation is 1.6. This may indicate that the limiting process in bud initiation is a chemical reaction, while that for root initiation is a physical one.

Additional information concerning temperature effects was obtained through the following experiment. Ten groups of test pieces were placed at 20° C. and 25° C. respectively. On each day, for a period of 10 days, a group was changed from 20° to 25° C. and another group from 25° to 20° C. Observations were made at the end of 12 days. The results are shown in figure 9. They indicate that temperature affects the initiation of buds and roots only at a particular stage in their development; *i.e.*, between the sixth and eighth days after isolation. Previous work (13) has shown

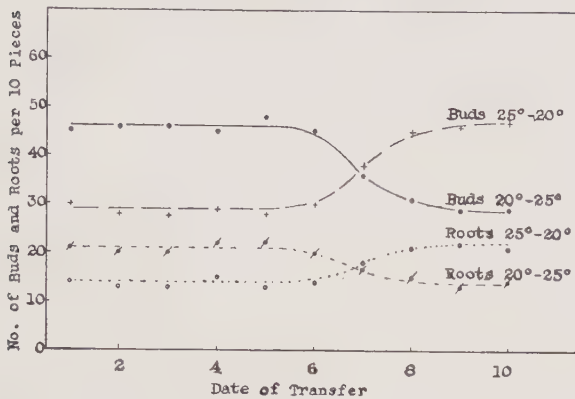


FIG. 9. Relation between time of temperature change and the initiation of buds and roots. The abscissa indicates time of transfer in days after beginning of experiment.

that this is the time when the meristematic regions are just beginning to organize into either buds or roots. It would appear, therefore, that temperature does not play an important rôle in the initial cell divisions, but is more effective in limiting the actual organizational processes.

A careful analysis of the effects of light on bud and root initiation was not attempted. A few experiments seemed to indicate that light stimulated bud production and inhibited root production. More exact experiments are necessary, however, to distinguish between the effects of light and the effects of temperature. To overcome any effects that light might have, all tests were made in the dark.

It was desirable to determine what effect the hydrogen-ion concentration of the medium around the test pieces would have on bud and root initiation. In these experiments, McILVAINE's standard buffer solutions (ranging from pH 2.2 to 8.0) were used instead of distilled water to moisten the sand in

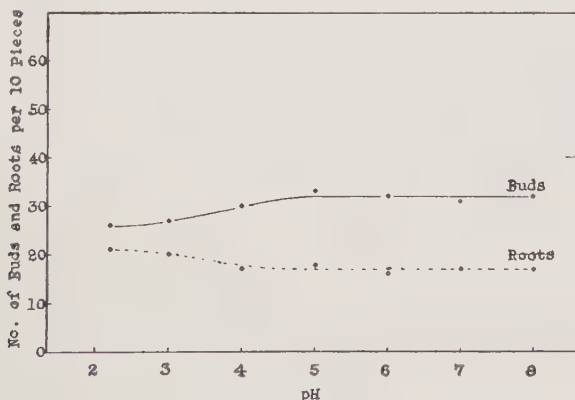


FIG. 10. Effect of hydrogen ion concentration on initiation of buds and roots.

the Petri dishes. These solutions (4), consisting of citric acid and potassium mono-hydrogen phosphate, are convenient to use because a wide range of pH may be obtained by varying the proportions of the two constituents. In every case the concentrations of the solutions were diluted one-tenth in order to prevent injury of the test pieces by high concentrations of salts. The hydrogen-ion concentration of the resulting solutions was checked with a quinhydrone electrode. No attempt was made to control the hydrogen-ion concentration of the tissue of the test pieces, and no determinations of tissue pH were made. The effects of these solutions on standard test pieces 3 mm. in length are shown in figure 10. Apparently the pH of the medium has little influence on the initiation of either buds or roots. This may be caused, in part, by a high buffer capacity of the tissue of the test piece. In the more acid solutions, there was a slight increase in number of roots and a slight decrease in number of buds. This could be accounted for most easily on the basis of an activation of native auxins by the acid (2).

After the method had been sufficiently standardized, it became of interest to determine the effect of specific chemical compounds on the initiation of buds and roots. In previous work, indoleacetic and naphthyleneacetic acids, in relatively high concentrations, were shown to be very effective in inhibiting the development of buds and in stimulating the development of roots (13). It was thus advisable to analyze further the effects of these compounds. Standard test pieces 3 mm. in length were treated with aqueous solutions of indoleacetic acid or naphthyleneacetic acid ranging in concentrations from 5.4×10^{-4} M to 5.4×10^{-14} M. Ten ml. of these solutions were added to the sand in Petri dishes. Since there were ten test pieces per dish, each piece had a maximum volume of 1 ml. of solution at its disposal, although only a small portion of this amount was probably absorbed. Lanolin mixtures of each of these substances were also applied to the cut surfaces of test pieces in concentrations ranging from 5.4×10^{-2} M to 5.4×10^{-7} M. These two different means of application gave essentially the same results. The effects of aqueous solutions of naphthyleneacetic acid are shown in figure 11. In the higher concentrations there is apparently a stimulation of root initiation and an inhibition of bud initiation (figure 3). No evidence was obtained for bud stimulation at any concentration over the wide range employed. The lower concentrations of naphthyleneacetic acid are more effective in bud inhibition than they are in root stimulation. Indoleacetic acid did not prove very effective, presumably because it was inactivated. The inactivation may have been caused by oxidizing enzymes from the test pieces, microorganisms, inorganic constituents of the sand, or a combination of these factors. At any rate, the "bud factor" appears to have no direct relation to the auxins; but it may be inactivated by them, or at least ineffective in their presence.

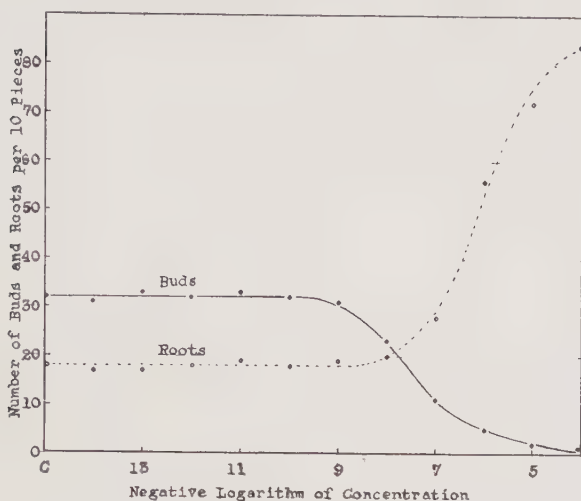


FIG. 11. The relationship of aqueous solutions of naphthyleneacetic acid to the initiation of buds and roots. The abscissa indicates the concentration in grams per liter. C, control.

When small isolated pieces of various tissues of the root (xylem only, phloem only, periderm plus phloem, etc.) were treated with relatively high concentrations of naphthyleneacetic acid, roots were initiated from all of the tissues. The main cambial region and the region of the phellogen were the most active in this respect. Root traces were not included in any of these pieces.

A number of standard test pieces, 3 mm. in length, were divided into twelve lots. Each day, for 12 days, a different lot was treated with a 1 per cent. lanolin mixture of naphthyleneacetic acid. Each lot thus received but one application. Observations were made after 20 days. The results are shown in figure 12. Apparently the number of roots initiated is about the same regardless of the time of application of naphthyleneacetic acid. The number of buds, however, is most affected if the treatment is made during the first four days after isolation. The application of naphthyleneacetic acid after this time was apparently too late to inhibit the initiation. This period between 5 and 7 days is the time when meristematic areas are just beginning to organize into either a bud or a root. Thus after a bud is once organized, it will not change over into a root although root initials may develop at the base of the bud. Gross observations of buds from pieces treated 6 days and more after isolation substantiate this conclusion (compare the results of the experiment shown in figure 7 in this regard). Between the fifth and eighth days, in this experiment, there was a maximum decrease in the number of buds on the test pieces isolated from the lower portions of the segments. This might indicate that the native auxins (or other bud

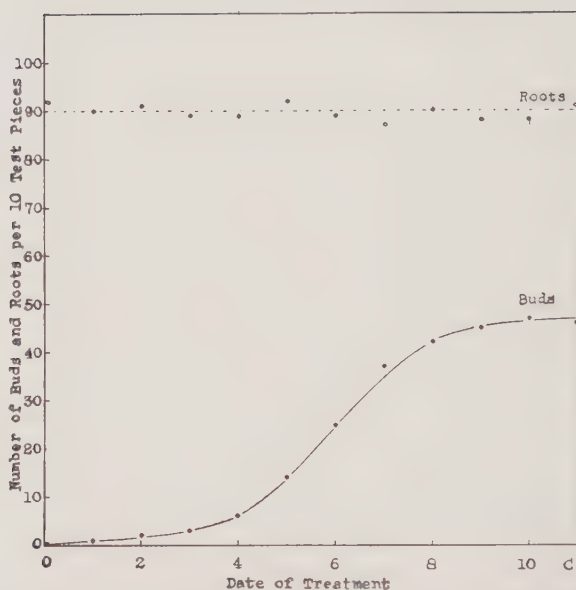


FIG. 12. The relation of time of application of a 1 per cent. lanolin mixture of naphthyleneacetic acid to the initiation of buds and roots. The abscissa indicates time of treatment in days after start of experiment. C, control.

inhibitors) do not start to accumulate in the lower regions of transverse segments of the root until about 4 days after isolation.

In the experiments reported in table I and in figures 5, 6, 10, and 11, the maximum number of buds formed per ten test pieces, 6 mm. in diameter and 3 mm. in length, is near 32; while for the experiments reported in figures 7, 8, 9, and 12, the maximum number is near 46. This is attributable to the fact that horseradish roots from different sources were used for these two different groups of experiments. The higher maximum was obtained on test pieces from roots purchased in the fall; the lower maximum was obtained on test pieces from roots purchased in the winter. Thus it is shown, in an indirect fashion, that the past history of the root is an important factor in determining the amount of regeneration.

After some of the effects of auxin-like compounds had been ascertained, the effects of other pure substances were determined. A number of inorganic and organic compounds were applied over a wide range of concentrations as aqueous solutions to the sand in the Petri dishes. Standard 1-mm. or 3-mm. test pieces were used in testing these substances at 25° C. in the dark. The following is a list of the materials tried:

Ammonium sulphate	Calcium nitrate
Ascorbic acid	Chloral hydrate
Calcium chloride	Citric acid

Cupric sulphate	Pangestin
Dioxane	Papain
Eosin	Potassium chloride
Ethyl alcohol	Potassium cyanide
Ethylene	Potassium di-hydrogen phosphate
Ethylene chlorohydrin	Potassium nitrate
Ferric sulphate	Potassium permanganate
Ferrous sulphate	Saliva
Formaldehyde	Sodium taurocholate
Glycine	Theelin
Hematoxylin	Theelol
Hydrogen peroxide	Thiourea
Inositol	Tyrosine
Magnesium sulphate	Urea
Maleic acid	Urine
Manganous sulphate	Vitamin B ₁ (thiamin)
Methyl amine	Vitamin B ₂
Methylene blue	Yeast extract (aqueous)
Nicotinic acid	Zinc sulphate

In general, high concentrations were toxic, but other than this, no marked effects were produced. Thus it is probable that none of these substances alone limited the initiating processes.

Preliminary extracts from various tissues of the horseradish root have given some indication of being capable of stimulating bud initiation. These extracts were tested on standard 1-mm. pieces and at least four different concentrations of each extract were used; the highest concentration was usually toxic. The following is an outline of the extracts tried:

- I. Inner tissues
 1. Ether extract
 2. Cell sap
- II. Outer and inner tissues
 1. Water extract
 2. Fraction of water extract soluble in 70 per cent. alcohol
 3. Fraction of water extract insoluble in 70 per cent. alcohol
- III. Inner tissues from roots allowed to regenerate 6 days
 1. Ether extract
 2. Water extract
 3. Cell sap
 4. Water diffusate
 5. Water extract² following previous ether and water extracts

² These extracts stimulated bud initiation at least 300 per cent.

6. Cell sap from frozen tissue
 - a. Soluble fraction not passing through collodion membrane
 - b. Insoluble fraction not passing through collodion membrane
- IV. Outer tissues from roots allowed to regenerate 6 days
 1. Ether extract
 2. Water extract² following previous ether extract
 3. Cell sap from frozen tissue
 - a. Soluble fraction not passing through collodion membrane
 - b. Insoluble fraction not passing through collodion membrane
- V. Outer and inner tissues from roots allowed to regenerate 6 days
 1. Water extract
 2. Fraction of water extract soluble in 70 per cent. alcohol
 3. Fraction of water extract insoluble in 70 per cent. alcohol

One encounters many difficulties in trying to obtain active extracts since there may be many different types of inhibitors present, and inactivation of an extract may take place for many reasons. The results, however, indicate that the "bud factor" is a chemical entity. This may be more apparent than real since the extracts may be effective by destroying bud inhibitors (such as the auxins) and may be entirely independent of the "bud factor." Further work of this type is now in progress.

Discussion

From the experiments reported here, it is apparent that the various tissues of the horseradish root possess different capacities for influencing the regeneration processes. Buds and roots are initiated only in association with a lateral root trace. The outer portions of the root trace appear to be responsible for root initiation; for maximum bud initiation, however, both the inner and outer portions are necessary. PRIESTLEY and SWINGLE (15) review some work on sea kale in this regard. The xylem tissues of the root of this plant, when isolated, produce buds from any of the cut surfaces, but do not form roots. The extra-cambial tissue, on the other hand, produces roots, but no buds. Thus these two species of plants are similar in that the inner tissues contain factors for bud initiation, and the outer tissues, factors for root initiation. They differ in that the horseradish regenerates only in association with a root trace, while any of the tissues of the sea kale root may regenerate. Furthermore, the horseradish requires factors that are present in the outer tissues of a root trace for maximum bud initiation.

The analysis of polarity phenomena in the horseradish indicates that in the dormant root there is no polar distribution of factors that cause either root or bud initiation. If the root is placed in an environment favorable for regeneration, however, the factors causing root initiation seem to become

polarly distributed. The factors associated with bud initiation also apparently become polarly distributed; this might be interpreted as caused by the inactivation of bud-initiating factors in the lower portions of the root or its isolated segments. SCHWANITZ (18) has done some work on this subject. He reports that intact rhizomes of *Lathyrus pratensis* and *Agropyrum repens* show a marked polar development of buds when allowed to regenerate. If the rhizomes are cut into transverse segments at the beginning of the experiment, each piece produces as many buds as any other piece. If severed on successive days, a gradual establishment of polarity is shown by the differential amount of bud development of the transverse segments. Further evidence has been accumulated by JONES (11), who reports that when segments of sea kale roots 5 to 10 cm. in length are centrifuged at least 3 days (1000 r.p.m.) with the apex of the segments away from the center, buds are usually produced from the base of the segments as well as at the apex. If segments are centrifuged with the base away from the center, however, they regenerate in a normal manner, *i.e.*, buds at apex and roots at base. This might mean that certain bud-inhibiting substances (such as auxins) are not subject to polar distribution when under the influence of an opposing centrifugal force, and thus their diffuse distribution allows buds to form from the basal ends of the segments. JONES also reports that segments of sea kale roots, 2 mm. or less in length, produced buds on both ends, while longer segments produced buds only on the upper end. This might indicate that the longer segments have more bud inhibitors than the shorter. STOUGHTON and PLANT (20), using segments of sea kale roots 7 cm. in length, cut off 1 mm. of tissue from the base and apex every 5 days for a period of 8 weeks. They obtained buds on both ends of 25 per cent. of the segments. This work would indicate that bud-inhibiting substances were removed when the end tissues were removed. FISCHNICH (6) was able to produce buds from callus at the basal end of poplar cuttings, that would ordinarily have produced roots, by ringing the cutting above the callus. The ringing supposedly prevented the accumulation of auxins in the basal callus.

In the horseradish, temperature has more effect on bud initiation than on root initiation. Other workers have reported similar results in regard to the relation of temperature to bud growth. CLARK (3) has shown that the decapitated hypocotyls of seedling tomato plants do not form buds from the cut surface unless the temperature is above 20° C. SIMON (19) reported that the development of buds from the callus of poplar cuttings was better at 32° C. than it was at 26° C. A temperature of 18° C. resulted in the formation of very little callus and inhibited the development of buds. JONES (11) found that a local increase of temperature as little as 2° C. induced the formation of buds on the lower ends of sea kale segments where roots ordi-

narily would have developed. Temperature is thus an important factor in bud initiation.

The hydrogen-ion concentration of the medium had little effect on bud and root initiation in the horseradish. JONES (11) reports similar results for sea kale. BONNER's analysis (2) of the effects of hydrogen ions on the *Avena* coleoptile shows that the effects of acids are produced through the activation of auxins. RIEHM's results (17) are a little difficult to understand in this regard. When he floated leaf fragments of *Cardamine pratensis* on a solution of KH_2PO_4 , buds were produced in 3 days and roots in 5 days. When the leaf fragments were floated on K_2HPO_4 , however, roots were formed in 3 days, but buds were not formed until after 14 days.

In the horseradish, the application of naphthyleneacetic acid over a wide range of concentrations inhibits the initiation of buds and stimulates the initiation of roots. STOUGHTON and PLANT (20) obtained similar results with sea kale roots by using relatively high concentrations of indoleacetic acid. WENT (24) also reports similar results with the roots of *Oenothera macrosiphon*. Thus it seems that, in certain plants at least, the initiation of buds from roots is inhibited by auxin-like compounds, while the initiation of roots is stimulated. The effects of these compounds on bud initiation from stems, however, are not so consistent. LINK and EGGERS (14) report inhibition of bud initiation from decapitated flax hypocotyls by application of relatively high concentrations of indoleacetic acid. On the other hand, GREENLEAF (8) reports a stimulation of bud initiation from the callus of decapitated tobacco plants through the use of a 1 per cent. indoleacetic acid mixture in lanolin. Likewise, GOLDBERG (7) reports similar results for cabbage seedlings. BEAL (1) obtained axillary buds by treating decapitated stems of *Lilium harrisii* with relatively high concentrations of indoleacetic acid. A closely related species, *L. longiflorum*, produced only roots in response to the treatment. HOWARD (10) reports that decapitated kale plants produce both buds and roots when treated with indoleacetic acid, but the buds are formed later than the roots—presumably after the supply of indoleacetic acid is exhausted. The work of COOPER (5), STUART (21), and WENT (23) on the mobilizing action of auxin-like compounds may lead the way to a solution of these problems in the future.

Ascorbic acid, thiamin, ethylene, and other "active" compounds are not effective in stimulating the initiation of either buds or roots in the horseradish. Extracts, however, indicate that the "bud factor" is a chemical entity. A factor affecting the development of buds has been reported by WENT (22). It is probably formed in the roots, requires intact cells for its transfer, and functions in bud elongation in collaboration with auxin. WENT has named this factor "cauloalaine." Whether this factor is involved in bud initiation is not known.

Summary

1. A method of analyzing some of the factors associated with root and bud initiation, by the use of small isolated pieces of the horseradish root, is outlined. The results from more than 15,000 test pieces are reported.

2. The various tissues of the horseradish root apparently possess different capacities for influencing the regeneration processes. Root and bud initiation are confined to the lateral root traces. The outer tissues of the root trace are apparently responsible for root initiation; for maximum bud initiation, both outer and inner tissues are necessary.

3. The factor (or factors) in the inner tissues that stimulates bud initiation moves only along the root trace, and requires intact cells for its transfer.

4. The dormant root of the horseradish does not possess a polar distribution of either root or bud initiation factors. A polarity in root initiation is established only after the root is placed in a favorable environment for regeneration. An apparent polarity in bud initiation is established at the same time.

5. Variations in temperature have more effect on bud initiation than on root initiation. Between 15° C. and 25° C. the Q_{10} for bud initiation is about 2.8; that for root initiation is about 1.6. The optimum temperature for both bud and root initiation is about 26° C. Temperature produces its greatest effect just at the time the meristematic regions are organizing into buds or roots.

6. The hydrogen-ion concentration of the medium has little effect on either root or bud initiation.

7. Naphthyleneacetic acid, over a wide range of concentrations, inhibits bud initiation and stimulates root initiation. At no concentration is there any evidence of the stimulation of bud initiation. Indoleacetic acid was not very effective, presumably because it was inactivated. Relatively high concentrations of these substances are capable of inducing root initiation from isolated pieces of any living tissue of the root.

8. Numerous organic and inorganic compounds, including various vitamins and other "active" substances, had little effect on either root or bud initiation.

9. Tests with extracts of various parts of the horseradish root indicate that one of the factors present in the inner tissues that stimulates bud initiation is a chemical entity.

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